1933

The organic acid content of normal and pathological blood by the use of the quinhydrone electrode.

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"THE ORGANIC ACID CONTENT OF NORMAL AND PATHOLOGICAL BLOOD BY THE USE OF THE QUINHYDRONE ELECTRODE"

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HISTORICAL

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Organic acids in blood and urine have been the cause of much interest and investigation for the past several years. This interest has been due to the fact that in certain pathological conditions there has been found a decrease or increase from the normal amounts of organic acids. Peters, Bulger, Eisenman, and Lee, (11) have investigated as to whether definite relations exist between the amount of organic acid present and the presence of certain characteristic symptoms, and if the combination of the two lead to a positive proof of disease or pathological state. Among the organic acids which have been recognized as present in ordinary blood and urine, and which have been isolated under various conditions, are uric acid, some of the amino acids, lactic acid, hippuric acid, and the ketone acids.

Gamble (4) identified and reported on carbonic acid and bicarbonate in urine. Peters and Van Slyke (7) and Folin, Otto, and Denis (16) have listed uric acid, lactic acid, hippuric acid, some of the amino acids, and the ketone acids as present in blood. Only small amounts of these acids are present in normal blood and urine, and these amounts vary under certain conditions. For instance, Perlzweig and Delrue (1) and Peters, Bulger, Eisenman, and Lee (9) found that exercise increases the total acids of plasma in health and disease. Hyperventilation tetany, presumably because of the production of lactic acid, gave increased amounts according to Peters, Bulger, Eisenman, and Lee (9) in their work on blood plasma. Van Slyke and Palmer (2) on urine analysis, and Peters, Wakeman, Eisenman, and Lee (8), Peters, Bulger, Eisenman,

and Lee (12), and Rabinowitch (21) on urine and blood analysis found an increased organic acid content, the excess of acid probably due to the abnormal presence of b-hydroxybutyric acid and acetoacetic acid. Peters, Bulger, Eisenman, and Lee (10) and Beck, Field, and Adair (15) concluded that diabetes causes an increase of organic acid in urine, more than 50 millimols having been found in some cases. This high content is the result of the acetone bodies and acetoacetic and b-hydroxybutyric acids which are formed in diabetes. Rabinowitch (21) in his determination of the degrees of acidosis in diabetes mellitus arrived at similar results. Greenwald (5) and Palmer (13) on urine analysis, claimed an increase of organic acids in pneumonia. Blatherwick and Long (14,19) and McLaughlin and Blunt (20), carrying out research on urinary acidity, found that the addition of certain foods, notably prunes, cranberries, sour milk, and orange juice, gave a rise in total organic acids. Peters, Bulger, Eisenman, and Lee (11) found that certain infections increase the organic acid content of plasma. Peters, Wakeman, Eisenman, and Lee (8), and Peters, Bulger, Eisenman, and Lee (12) gave evidence that organic acids increase in cases of nephritis, but they are doubtful as to the reason and nature of the acid causing the increase. Peters and Van Slyke (7) question Peter's work on uremia (8,12) because of his failure to determine serum sulfate which might have led to an exaggeration of the organic acid concentration. Peters and Van Slyke (7) believe it possible that part of the deficit assigned by Peters (8,12) to organic acid really represents base combined with inorganic sulfur.

Peters, Wakeman, Eisenman, and Lee (8) have found that the base combined with organic acid in the serum of normal, resting

individuals is about 10 millimols of equivalent base and is rarely above 10. Uric acid and the amino acids seem to make up a rather negligible part of this total. Perlzweig and Delrue (1) found in normal, blood, serum, and plasma between 5 and 8 milliequivalents per liter.

It is the object of this paper to submit to trial the method of Perlzweig and Delrue (1), and to find the relative increase or decrease of the organic acids in blood under various conditions.

METHODS

METHODS

The determination of total organic acids in urine is a relatively simple process, using the method of Van Slyke and Palmer (2,5). They precipitate the carbonates and phosphates with solid calcium hydroxide, and filter out the precipitate. Two drops of phenolphthalein are added to the filtrate and enough N hydrochloric acid is run in to give an acid medium. Then the indicator tropaeolin OO is introduced, and the solution is again titrated with N hydrochloric acid. This procedure is based on the difference in titration from the turning point of phenolphthalein to the turning point of the tropaeolin OO, or from a pH of 8.0 to a pH of 2.3.

The original method for the organic acid calculation in blood (6) was known as the indirect method. By determining the total base of the blood or plasma and subtracting from it the sum of the acids HCO_3, Cl, HPO_4, SO_4, and the base binding value of the proteins, the base binding value of the organic acid was estimated indirectly. This method was long, indirect, and often incorrect, so attempts were made to apply Van Slyke and Palmer's (2,5) method for urine to blood analysis. Perlzweig and Delrue (1) made repeated attempts to use the titration-indicator method employing thymol blue, bromphenol blue, and tropaeolin OO. The concentration of the blood acid was too small to give any end-points or indicator reaction, so they proposed a direct determination employing electrometric titration using a quinhydrone electrode instead of an indicator.

EXPERIMENTAL

EXPERIMENTAL

Apparatus and Reagents

Quinhydrone-

A solution of 100 grams of ferric ammonium sulfate dissolved in 300 c.c. of water at 65° C. is poured into a solution of 25 grams of hydroquinone in 500 c.c. of water. This mixture is cooled in ice, the quinhydrone precipitating as dark green crystals of needlelike structure. The crystals are collected in a suction filter, redissolved and recrystallized from distilled water, and are dried. The quinhydrone is relatively pure when finally dried and collected.

Quinhydrone electrode-

A gold wire electrode which dips into the titrated solution and which must be completely covered by the solution. The electrode is cleaned frequently with a one to one solution of nitric acid and water.

Calomel cell-

A standard saturated calomel cell, made of redistilled mercury, a paste of calomel and mercury, and a saturated solution of potassium chloride and calomel.

KCl--agar bridge-

A glass U-tube is filled with a hot solution of 3 grams of agar-agar and 30 grams of potassium chloride dissolved in 100 cc.

of water. This solution jells on cooling and forms the KCl-agar bridge which is used to connect the calomel cell with the solution being titrated. This bridge must be free from air bubbles, as any air would increase the resistance and would give incorrect voltage for the sample. This solid bridge also prevents any siphoning of the sample into the calomel cell. Between the titrations the bridge is kept in a saturated solution of potassium chloride. This KCl-agar bridge has a high conductivity. For a description of the set-up, refer to Figure 6.

Buffers-

A set of Clark and Lubs buffers (18) are used. Various reagents are mixed in definite proportions to give the different pH values. There are four sets, the 0.2M acid potassium o-phthalate and 0.2N hydrochloric acid group, the 0.2M acid potassium o-phthalate and 0.2N sodium hydroxide group, the 0.2M acid potassium phosphate and 0.2N sodium hydroxide group, and the 0.2M boric acid-potassium chloride and 0.2N sodium hydroxide group. For the buffer solution of pH 4.6, which is used as the buffer control for the blood samples, 50 cc. of 0.2M acid potassium phthalate and 12.15 cc. of 0.2N sodium hydroxide are diluted to 200 cc. This set of Clark and Lubs buffers gives more accurate results than the Michaelis standard acetate buffer suggested by Perlzweig and Delrue (1).

M/10 sodium chloride-

This is used as the control. Dissolve 5.846 grams of chemically pure sodium chloride in distilled water, and make up to a liter of solution.

N/10 sodium hydroxide-

Dissolve 4.001 grams of sodium hydroxide in distilled water, and make up to a liter of solution. The carbon dioxide present in the sodium hydroxide solution is precipitated with a 10% barium chloride solution, and the barium carbonate is filtered off. The sodium hydroxide is standardized against a N/10 oxalic acid solution using phenolphthalein as the indicator.

Copper sulfate solution-

Dissolve 10 grams of chemically pure copper sulfate in 100 cc. of distilled water. This gives a 10% solution.

N. hydrochloric acid-

Dissolve 80 to 90 cc. of concentrated hydrochloric acid in distilled water, and dilute to a liter of solution. The hydrochloric acid is standardized against the standard sodium hydroxide, using methyl orange as the indicator.

PROCEDURE

PROCEDURE

The blood is centrifuged, and 5 c.c. of the blood plasma are measured with a pipette. The proteins in the plasma are precipitated with glacial phosphoric acid ($HPO_3 = HaPO_3$), about 5 c.c. of a 25% water solution having been found by Hiller and Van Slyke (17) to be a sufficient amount for a complete precipitation of the proteins in the sample. The phosphoric acid must be prepared as needed, and if kept cool will last at the most only two or three days. Approximately 5 to 10 c.c. of a 10% copper sulfate solution are added to precipitate the blood sugar. Enough solid calcium hydroxide (1.8 to 2 grams) is added to make the solution alkaline, the calcium hydroxide serving to precipitate the carbonates, phosphates, and oxalates. The alkaline sample is then evaporated on a water bath to about one half its volume, and it is filtered again as more carbonates, phosphates, and oxalates precipitate on heating. Two drops of methyl orange indicator are added, and the medium is made acid with N hydrochloric acid. The quinhydrone is added, a few milligrams being enough for each determination, and the sample is placed in a water bath at 25° C. The quinhydrone electrode is placed in the solution, and care must be taken that the gold wire of the electrode is completely covered by the solution. By means of a saturated potassium chloride-agar bridge, a saturated calomel cell (suspended in the same water bath) is connected to the sample, and the electrode and calomel cell are then connected to the Leeds and Northrup indicator (see Figure 6). More N hydrochloric acid is added until a pH of 2.0 to 2.2 is reached, and the sample is then titrated with a carbon dioxide free N/10 sodium hydroxide solution, using

a micro-burette. Small portions of the sodium hydroxide are run in, the circuit is closed by tapping the electrode key of the Leeds and Northrup indicator, and the deflection of the galvanometer observed. The potentiometer is adjusted until there is no deflection in the galvanometer upon closing the circuit, and the voltage is recorded. The titration is carried out slightly beyond the change of polarity, or to a pH of 8.0 to 8.5.

A M/10 sodium chloride solution is used as a control. It is treated simultaneously with and exactly as the blood sample. The buffer solution which is used as a check for the calomel cell and from whose pH is obtained the pH of the control and sample, is a Clark and Lubs (18) acid potassium phthalate-sodium hydroxide solution of pH 4.6. Parnas and Delrue (1) suggest the use of a Michaelis standard acetate buffer of pH 4.62, but this buffer, although an excellent one, loses acetic acid very easily and changes its pH too readily.

The pH values of the controls and samples may be obtained from the value for the buffer from the following equation—

$$pH = 4.62 + \frac{E_0 - E_1}{RT/F}$$

where E_0 is the observed potential in volts of the buffer of pH 4.62 and E_1 is the observed potential in volts of the titrated control or sample. The RT/F factor for 25° C. is 0.0591, and is constant, (0.0001984 × 273 + t). Van Slyke and Palmer (2,6) made a correction for the creatine and for creatinine, but for blood or plasma this correction is negligible.

Curves for the blood plasma and for the sodium chloride control are plotted on graph paper, the pH values plotted against the

amount of N/10 sodium hydroxide used. The amount of alkali necessary to bring the control to a pH of 8 is subtracted from the amount of alkali necessary to bring the blood sample to a pH of 8, giving the milliequivalents of organic acid in 5 c.c. of blood. This is corrected to milliequivalents of acid per liter of blood by multiplying by 200 and, corrected to milliequivalents of normal alkali by dividing by 10. The final answer is in milliequivalents of normal acid per liter of blood.

TABLES

Table 1.

Typical titration of a control and of a normal blood sample.

Control—

.2 c.c.N/10 NaOH	volt = -.326	pH = 2.15
1.0 c.c.N/10 NaOH	volt = -.323	pH = 2.20
2.0 c.c.N/10 NaOH	volt = -.318	pH = 2.29
3.0 c.c.N/10 NaOH	volt = -.312	pH = 2.39
4.0 c.c.N/10 NaOH	volt = -.302	pH = 2.56
5.0 c.c.N/10 NaOH	volt = -.2855	pH = 2.84
6.0 c.c.N/10 NaOH	volt = -.249	pH = 3.46
6.4 c.c.N/10 NaOH	volt = -.061	pH = 6.63
6.6 c.c.N/10 NaOH	volt = +.065	pH = 9.07

Blood sample—

.2 c.c.N/10 NaOH	volt = -.326	pH = 2.15
1.0 c.c.N/10 NaOH	volt = -.325	pH = 2.20
2.0 c.c.N/10 NaOH	volt = -.318	pH = 2.29
3.0 c.c.N/10 NaOH	volt = -.312	pH = 2.39
4.2 c.c.N/10 NaOH	volt = -.300	pH = 2.59
5.1 c.c.N/10 NaOH	volt = -.287	pH = 2.81
6.0 c.c.N/10 NaOH	volt = -.251	pH = 3.42
6.5 c.c.N/10 NaOH	volt = -.212	pH = 4.25
6.6 c.c.N/10 NaOH	volt = -.119	pH = 5.65
6.9 c.c.N/10 NaOH	volt = +.020	pH = 8.00

Table 2.

Typical titration of a control and of a blood sample from normal pregnancy.

Control—

.2 c.c.N/10 NaOH	volt = -.325	pH = 2.17
1.0 c.c.N/10 NaOH	volt = -.319	pH = 2.27
2.0 c.c.N/10 NaOH	volt = -.309	pH = 2.44
3.0 c.c.N/10 NaOH	volt = -.294	pH = 2.70
3.5 c.c.N/10 NaOH	volt = -.280	pH = 2.95
4.0 c.c.N/10 NaOH	volt = -.251	pH = 3.42
4.5 c.c.N/10 NaOH	volt = -.128	pH = 5.50
5.0 c.c.N/10 NaOH	volt = +.020	pH = 8.00

Blood sample—

.2 c.c.N/10 NaOH	volt = -.325	pH = 2.17
1.5 c.c.N/10 NaOH	volt = -.315	pH = 2.34
2.5 c.c.N/10 NaOH	volt = -.308	pH = 2.46
3.1 c.c.N/10 NaOH	volt = -.300	pH = 2.59
4.1 c.c.N/10 NaOH	volt = -.279	pH = 2.95
5.3 c.c.N/10 NaOH	volt = -.110	pH = 5.80
5.6 c.c.N/10 NaOH	volt = +.071	pH = 8.86

Table 3.

Typical titration of a control and of a blood sample from eclampsia.

Control--

.2 c.c.N/10 NaOH	volt = -.3265	pH = 2.15
1.0 c.c.N/10 NaOH	volt = -.3235	pH = 2.20
2.0 c.c.N/10 NaOH	volt = -.319	pH = 2.27
3.0 c.c.N/10 NaOH	volt = -.312	pH = 2.39
4.0 c.c.N/10 NaOH	volt = -.304	pH = 2.53
5.3 c.c.N/10 NaOH	volt = -.2866	pH = 2.85
6.0 c.c.N/10 NaOH	volt = -.249	pH = 3.46
6.4 c.c.N/10 NaOH	volt = -.109	pH = 5.82
6.7 c.c.N/10 NaOH	volt = 4.100	pH = 9.35

Blood sample--

.2 c.c.N/10 NaOH	volt = -.326	pH = 2.15
1.0 c.c.N/10 NaOH	volt = -.3235	pH = 2.20
2.5 c.c.N/10 NaOH	volt = -.317	pH = 2.30
2.7 c.c.N/10 NaOH	volt = -.316	pH = 2.33
3.3 c.c.N/10 NaOH	volt = -.312	pH = 2.39
4.5 c.c.N/10 NaOH	volt = -.301	pH = 2.58
5.3 c.c.N/10 NaOH	volt = -.288	pH = 2.79
6.1 c.c.N/10 NaOH	volt = -.258	pH = 3.30
6.5 c.c.N/10 NaOH	volt = -.200	pH = 4.28
6.7 c.c.N/10 NaOH	volt = -.146	pH = 5.19
7.1 c.c.N/10 NaOH	volt = +.048	pH = 8.48

Table 4.

Typical titration of a control and of a blood sample from tuberculosis.

Control--

.2 c.c.N/10 NaOH	volt = -.326	pH = 2.15
1.0 c.c.N/10 NaOH	volt = -.323	pH = 2.22
2.0 c.c.N/10 NaOH	volt = -.3195	pH = 2.26
3.0 c.c.N/10 NaOH	volt = -.314	pH = 2.36
4.0 c.c.N/10 NaOH	volt = -.308	pH = 2.46
5.0 c.c.N/10 NaOH	volt= -.300	pH = 2.59
6.0 c.c.N/10 NaOH	volt = -.288	pH = 2.80
7.0 c.c.N/10 NaOH	volt = -.269	pH = 3.12
8.0 c.c.N/10 NaOH	volt = -.171	pH = 4.77
8.3 c.c.N/10 NaOH	volt = -.017	pH = 7.37
8.35c.c.N/10 NaOH	volt = +.020	pH = 8.00

Blood sample--

.2 c.c.N/10 NaOH	volt = -.326	pH = 2.15
1.0 c.c.N/10 NaOH	volt = -.3235	pH = 2.20
2.0 c.c.N/10 NaOH	volt = -.321	pH = 2.24
3.0 c.c.N/10 NaOH	volt = -.318	pH = 2.29
4.0 c.c.N/10 NaOH	volt = -.313	pH = 2.37
5.0 c.c.N/10 NaOH	volt = -.308	pH = 2.46
6.1 c.c.N/10 NaOH	volt = -.300	pH = 2.59
7.0 c.c.N/10 NaOH	volt = -.290	pH = 2.76
8.0 c.c.N/10 NaOH	volt = -.276	pH = 3.00
9.0 c.c.N/10 NaOH	volt = -.237	pH = 3.66
9.5 c.c.N/10 NaOH	volt = -.120	pH = 5.63
9.8 c.c.N/10 NaOH	volt = +.020	pH = 8.00

Table 6.

Typical titration of a control and of a blood sample from diabetes mellitus.

Control—

.2 c.c.N/10 NaOH	volt = -.3255	pH = 2.16
1.0 c.c.N/10 NaOH	volt = -.322	pH = 2.22
2.0 c.c.N/10 NaOH	volt = -.318	pH = 2.29
3.0 c.c.N/10 NaOH	volt = -.313	pH = 2.37
4.0 c.c.N/10 NaOH	volt = -.307	pH = 2.48
5.0 c.c.N/10 NaOH	volt = -.299	pH = 2.64
6.0 c.c.N/10 NaOH	volt = -.288	pH = 2.80
7.0 c.c.N/10 NaOH	volt = -.268	pH = 3.47
8.0 c.c.N/10 NaOH	volt = -.186	pH = 5.02
8.2 c.c.N/10 NaOH	volt = -.028	pH = 7.19
8.3 c.c.N/10 NaOH	volt = +.040	pH = 8.34

Blood Sample—

.2 c.c.N/10 NaOH	volt = -.326	pH = 2.17
1.0 c.c.N/10 NaOH	volt = -.324	pH = 2.19
2.0 c.c.N/10 NaOH	volt = -.321	pH = 2.24
3.0 c.c.N/10 NaOH	volt = -.318	pH = 2.29
4.0 c.c.N/10 NaOH	volt = -.313	pH = 2.37
5.0 c.c.N/10 NaOH	volt = -.307	pH = 2.48
6.0 c.c.N/10 NaOH	volt = -.300	pH = 2.59
7.0 c.c.N/10 NaOH	volt = -.290	pH = 2.76
8.0 c.c.N/10 NaOH	volt = -.274	pH = 3.03
9.1 c.c.N/10 NaOH	volt = -.224	pH = 3.98
9.6 c.c.N/10 NaOH	volt = -.100	pH = 5.97
9.8 c.c.N/10 NaOH	volt = +.020	pH = 8.00

Table 6.

Normal Bloods

Sample	Date	Milliequivalents of organic acid	
1	1/30/33	8	
2	2/3/33	8	
3	2/15/33	6	
4	2/15/33	10	
5	2/27/33	4	
6	3/6/33	4	
7	3/20/33	8	
8	3/20/33	8	
9	3/24/33	10	after moderate exercise
10	3/24/33	11	after more severe exercise

Table 7.

Normal Pregnancy

Blood sample	Date	Milliequivalents of organic acid
E. G.	3/13/33	8
E. H.	3/15/33	10
B.	4/28/33	10
E.	"	6
W.	"	4

Table 8.

Eclampsia

Blood sample	Date	Milliequivalents of organic acid
R. L.	2/17/33	8
G. B.	2/17/33	8
M. G.	3/3/33	4
I. W.	3/3/33	10

Table 9.

Pulmonary Tuberculosis

Blood sample	Date	Milliequivalents of organic acid
J. S.	4/21/33	32
E. L.	"	29
A. W.	"	33
M. H.	4/22/33	14
A. C.	"	27
H. C.	"	25
J. H.	"	21
R. L.	"	9
C. S.	"	37

Table 10.

Diabetes Mellitus

Sample	Date	Condition	Milliequivalents of organic acid	Blood sugar	
E. S.	3/17/33	mod. severe	30	142	no insulin
M. D.	3/18/33	mod. severe acidosis	20	300	insulin
C. H.	3/20/33	mod. severe	56	210	"
I. C.	3/20/33	mild	30	250	"
A. H.	3/20/33	mod. severe acidosis	26	250	"
L. B.	3/27/33	mild	30	162	no insulin
W. B.	4/10/33	mod.severe	44	400	insulin
R. P.	4/24/33	mod. severe	26	200	"

Table 11.

Diabetes Mellitus

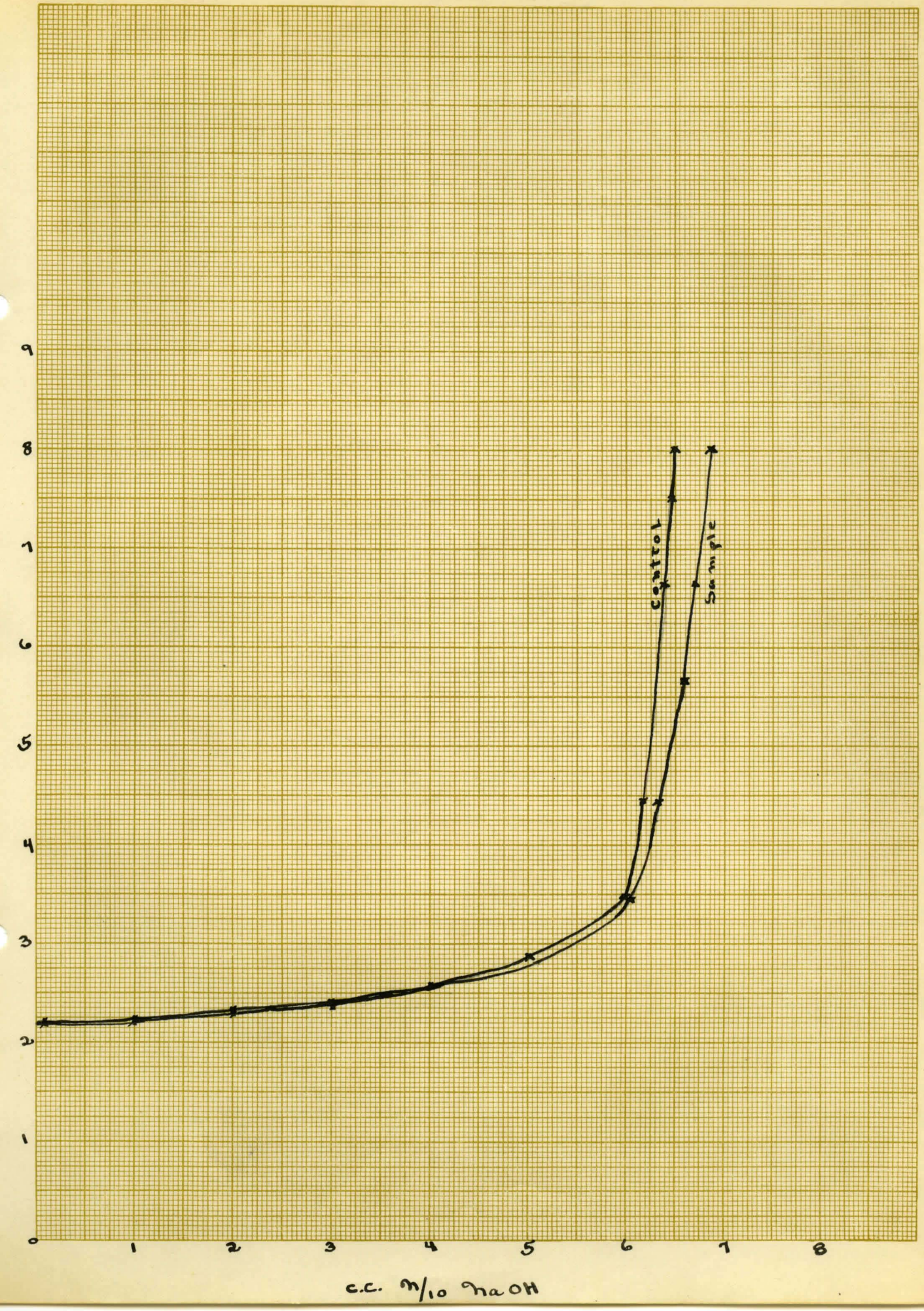
Sample	Date	Condition	Milliequivalents of organic acid	Blood sugar	
E. P.	3/20/33	acidosis	43	222	insulin
	3/27/33		34	181	
E. B.	3/27/33	mod. severe	52	286	insulin
	4/3/33		30	222	
	4/10/33		4	250	
	4/17/33		9	220	
G. C.	3/27/33	mild	40	222	insulin
	4/3/33		32	142	
	4/10/33		16	142	
	4/17/33		13	140	
A. I.	3/27/33	mild	30	200	no insulin
	4/3/33		35	106	
	4/10/33		22	200	
	4/17/33		31	220	
	4/24/33		44	240	
M. C.	3/31/33	mild	46	166	insulin
	4/3/33		34	196	
	4/9/33		8	138	
M. T.	4/7/33	mod. severe	8	333	insulin
	4/17/33		15	300	
E. L.	4/10/33	mild	5	222	insulin
	4/17/33		5	220	

FIGURES

Form M-330

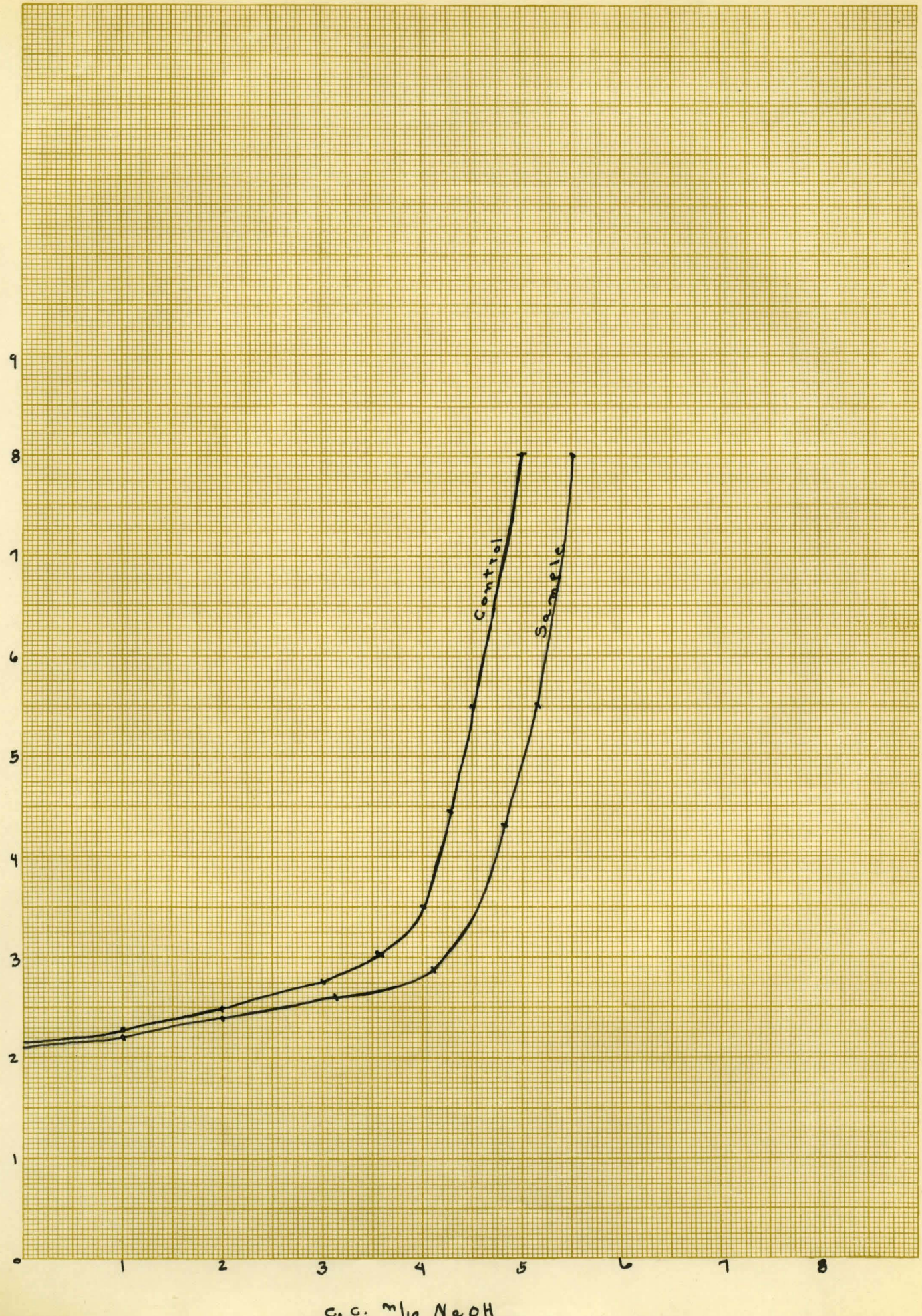
Figure 1.

(See Table 1.)



Form M-330

Figure 2
(See Table 2)



Form M-330

Figure 3
(See Table 3)

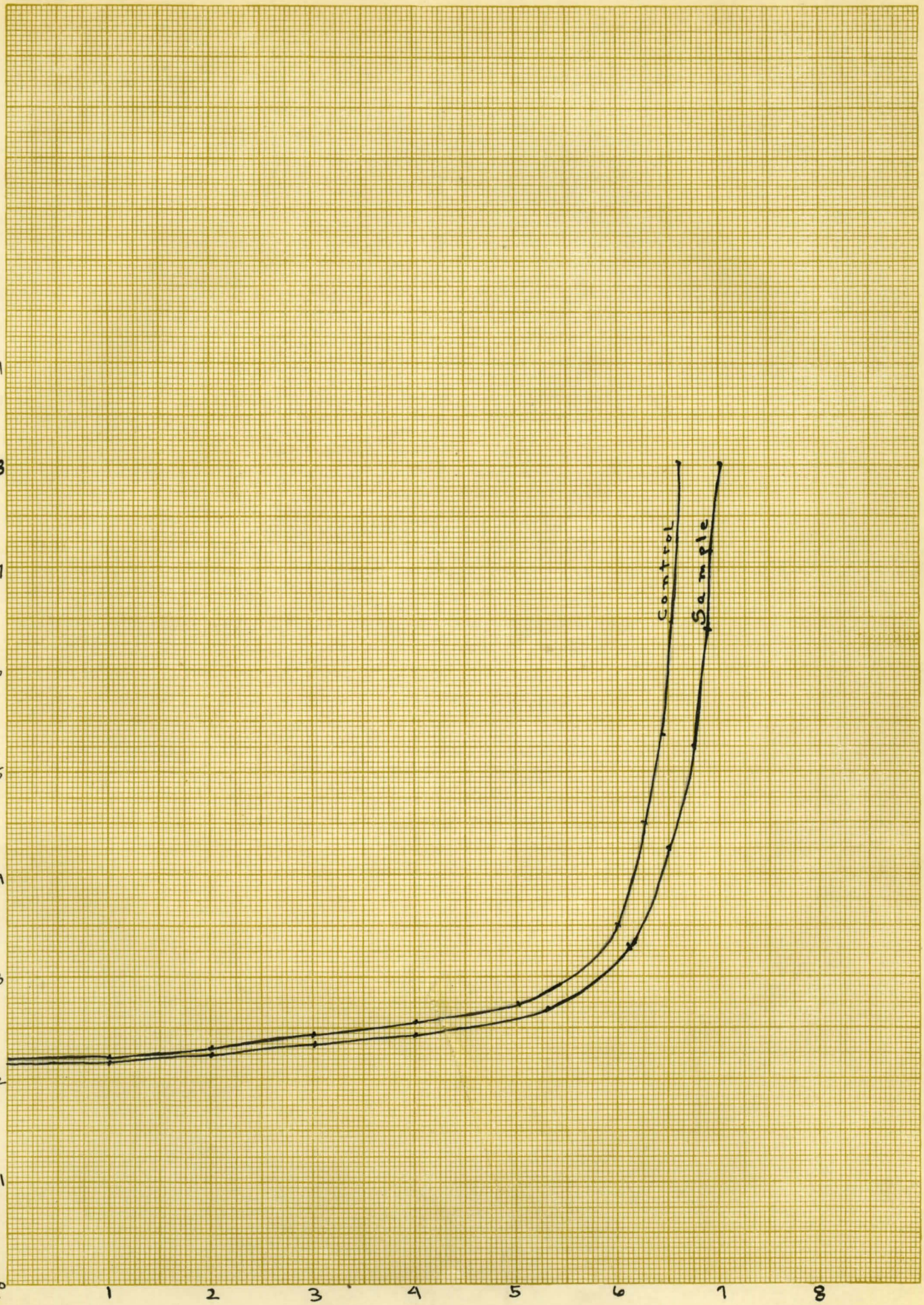


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Figure 4
(See Table 4)

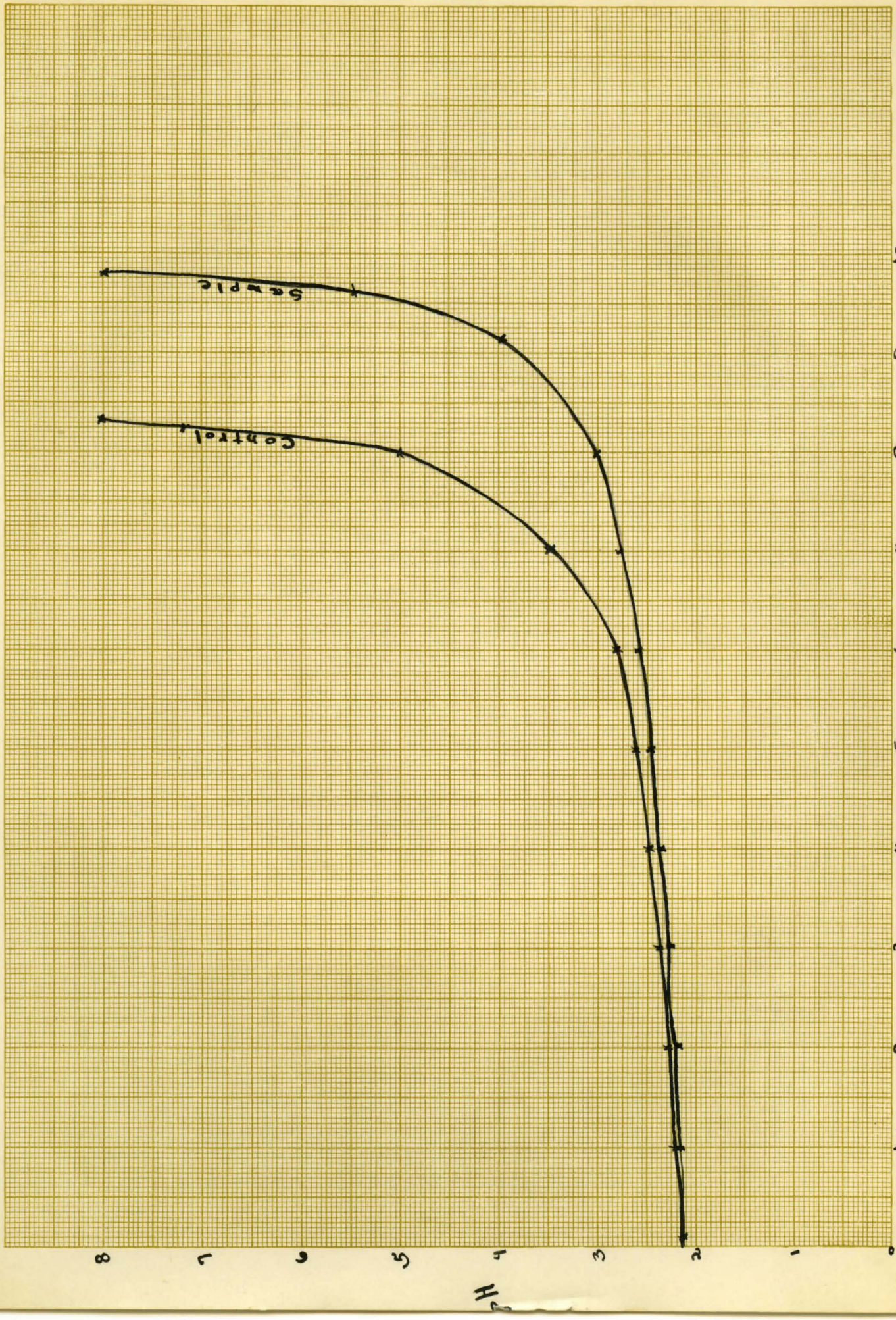

Figure 5
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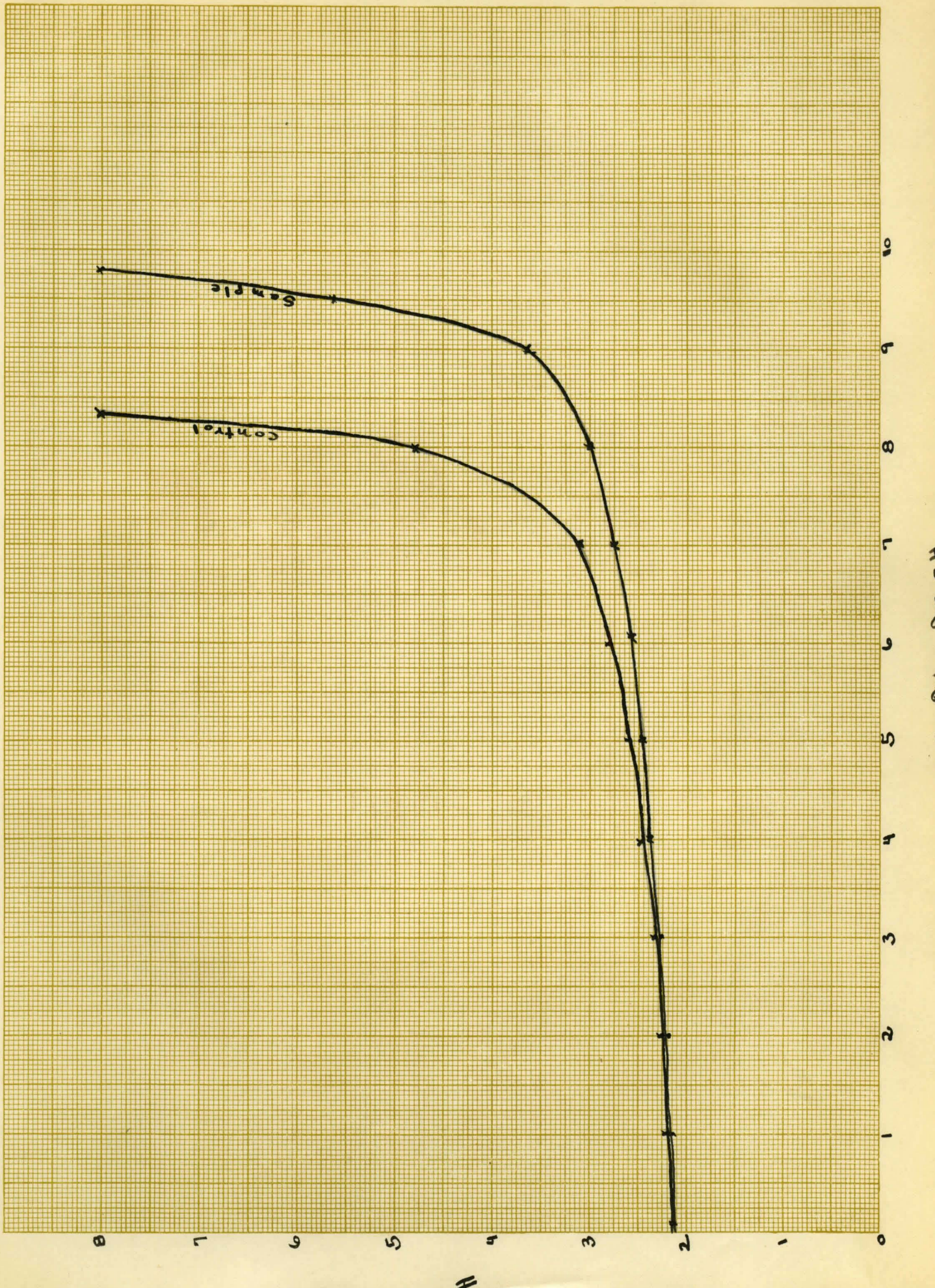

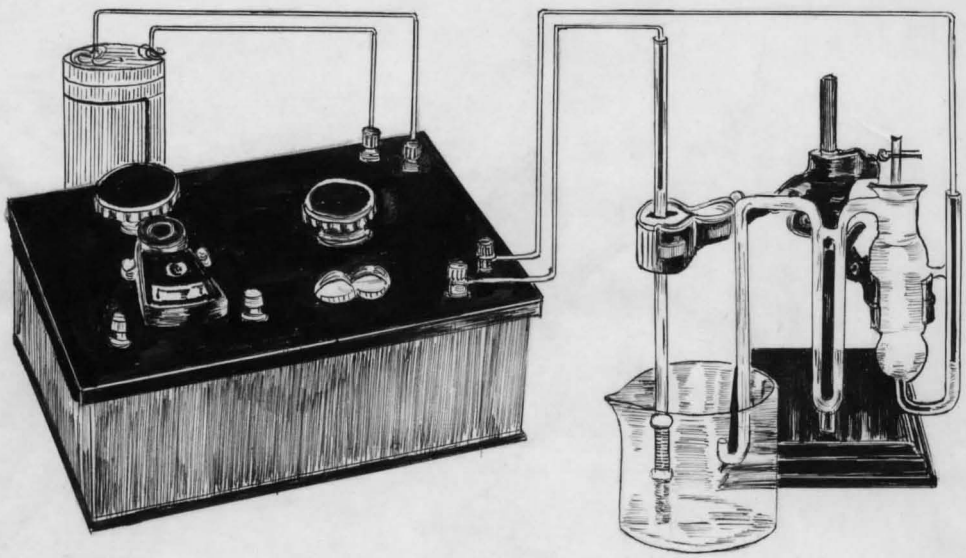

Figure 6

DISCUSSION

DISCUSSION

With the exception of the work reported by Perlzweig and Delrue (1), all the determinations of the organic acid content of blood previously reported have been made by the indirect method. The data obtained by use of the quinhydrone electrode method in this investigation is compared in the following pages with those values obtained by the indirect method, wherever the same types of diseases were studied. In the cases where no comparable data is available, the values are interpreted on the basis of the known facts regarding acid-base balance in the human body. It is necessary, therefore, at this point to give a short resume of these known facts as an aid in the interpretation of the values given in Tables 6 to 11.

The basic elements of the blood and tissues consist almost entirely of sodium, potassium, calcium, and magnesium. The minute amounts of the other basic material are negligible. At reactions so nearly neutral as those which prevail in the body, the alkali metals can exist only in combination with acids as neutral salts. Consequently the sum of the acid equivalents must equal that of the bases. Peters and Van Slyke (7) have expressed this relation graphically for average plasma in the following manner:—

$B = Na + K + Ca + Mg = Cl + HCO_3 + PO_4 + protein + SO_4$ and organic acid anions.

155 = 142 + 5 + 5 + 3 = 103 + 28 + 2 + 16 + 6

(The numbers under the symbols indicate milliequivalents per liter of plasma.)

Thus B is equal to the total base and is the sum of the milliequivalents of Na, K, Ca, and Mg. In normal human serum the

concentration of total base varies from 150 to 160 milliequivalents (8). Therefore, the bases determine the electrolyte content of the plasma and any change in the anion content is at the expense of one or more of the other anions. The individual anions are subject to gross variations in pathological conditions. Loss of HCl by vomiting may cause half the Cl to disappear and be replaced by HCO_3 and other anions. Short vigorous exercise may replace half the bicarbonate with lactate. Increase in CO_2 tension may cause an increase in HCO_3 accompanied by a fall in Cl (35). Bicarbonate reduction caused by hyperventilation may be balanced by increase in Cl or organic acid anions (9). Organic acids formed during starvation (37) or diabetic acidosis replace HCO_3 and Cl (10). Ultimately, if the metabolic cause of the electrolyte disturbance ceases, the abnormal organic anions are removed by combustion or excretion, Cl and HCO_3 being retained in their places, until normal ionic composition of the body fluids is reestablished.

Normal Blood—

The organic acid content of seven samples of blood taken from normal human individuals at rest was found to be 4 to 8 milliequivalents per liter of blood (Table 6). Only one of the seven individuals had an organic acid content higher than 8 milliequivalents. This is in agreement with the findings of Perlzweig and Delrue (1) who reported 5 to 8 milliequivalents.

Normal Blood after Exercise—

Perlzweig and Delrue found that after exercise the organic acid content of the blood of normal individuals was increased. This also was verified by the analysis of the blood of one normal individual after moderate exercise and again after more severe exercise. The amounts

found were 10 and 11 milliequivalents. These values were not so high as some reported, but the severity and prolongation of the exercise are the determining factors. It is well known that muscular exercise or excessive tissue activity causes an increase of lactic acid in the tissues and the blood, and produces an organic acid excess which is usually neutralised by base derived from bicarbonate. A lactic acid acidosis, therefore, results. The more severe the exercise the greater is the bicarbonate deficit.

Normal Pregnancy—

Blood specimens from five normal pregnant women were found to contain 4 to 10 milliequivalents of organic acid. These blood specimens were taken from young primiparas who had no clinical symptoms of toxemia of pregnancy. It has been claimed by Bökelman and Bock (30,31), Schmidt (32) and others that during pregnancy the concentration of ketone acids in the blood increases. This, they believe, is the cause of serum bicarbonate deficit regularly found in pregnant women. Gard and Peters (33) have shown that the low bicarbonate is not due to accumulation of organic acid, but to deficiency of base in the serum. The values for ketone bodies in the blood of pregnant women which are reported by Bökelman and Bock only occasionally exceed those of the non-pregnant. This is in agreement with the findings shown in Table 7. These values are practically the same as found in non-pregnant individuals.

Toxemias of Pregnancy—

In toxemias of pregnancy and vomiting of pregnancy, high values for organic acid concentration in blood have at times been observed. Usually they could be referred to starvation or the exercise of convulsions. Zweifel and Schaller (34) demonstrated an increase in

the blood lactic acid of eclamptic patients immediately after convulsive seizures; but this rapidly fell to normal after the convulsions were over.

The four specimens of blood (Table 8) from eclamptic patients analyzed in this investigation were found to contain no more organic acid than specimens from normal pregnant women. These bloods were taken when the patients entered the hospital. All four patients had high blood pressures and definite clinical symptoms typical of eclamptics. However, none of these women were afflicted with vomiting, and none reached the state of convulsions, which, in the light of previous work, may account for the normal values obtained. It seems evident, therefore, that it is only in the advanced stages of eclampsia that increased organic acids may be found. During the progress of this investigation, no specimens were obtainable from patients in such advanced stages.

Tuberculosis--

The analysis of specimens of blood from nine patients with pulmonary tuberculosis showed a marked increase in organic acid as determined by the quinhydrone electrode method (Table 9). Only two reports have been made on the blood acids in tuberculosis and both are concerned with lactic acid alone and not with the total organic acid. Valentin (28) claims that blood lactic acid is high in all conditions involving dyspnea. Jervell (29) observed fairly regular but moderate increases of lactic acid in the blood of patients with advanced pulmonary tuberculosis. That there is a disturbance of electrolyte metabolism in febrile infections has been reported by several investigators. Sunderman, Austin, and Cannack (36) have found low values for Cl in pulmonary tuberculosis and pleurisy with effusions. No doubt

the increase in organic anions was at the expense of Cl. Since malnutrition is so often associated with tuberculosis it seems reasonable to assume that the increase in organic acids is due to the effects of starvation. Muscle tissue presumably influences the metabolism of the body electrolytes more than any other type of tissue. The muscles comprise a greater proportion of the bulk of the organism than any other tissue; and in the autolysis of disease or inanition they appear to be involved first and most deeply.

Diabetes Mellitus--

In diabetic intoxications, the organic acid is greatly increased by the addition of b-hydroxybutyric and acetoacetic acids. Normally lactic acid, produced by tissue activity, can be oxidized, reconverted to glycogen in the tissues themselves, or transported to the liver for conversion. In diabetes the oxidation of lactic acid in tissues and possibly, its reconversion to tissue glycogen, is impaired or prevented, resulting in greater than normal amounts in the blood. It has been reported that uric acid has been found in increased amounts in diabetic blood. Rock, Field, and Adair (15) and Starr and Frits (38) have found in some cases of diabetes, acidosis due to organic acids other than b-hydroxybutyric and acetoacetic acids. It is not entirely certain that this was related directly to the diabetic condition, although Labbé, Bith and Nepveux (39) have reported similar phenomena. The latter prefer the total organic acid titration rather than direct determinations of the ketones, more on this account, because it permits the detection of acidosis due to other acids as well as that due to ketone bodies alone.

In the milder cases of diabetes without acidosis or severe polyuria the metabolism of base and its concentration in the serum are

not appreciably disturbed.(10) In severe diabetic acidosis it is not uncommon to find the serum base concentration low (10). Thus any increase in organic anions must replace some of the other acid anions. In the milder types of the disease, and in severe cases receiving an adequate diet and showing no evidence of edema or ketosis, the chlorides, as a rule, in the plasma are normal. However, in more severe cases of acidosis and coma there is loss of Cl; the base Na combining with the organic anions. Since protein metabolism is usually normal in diabetes, the anions replaced must be HCO_3 and PO_4. That this is the case is verified by reports of low serum phosphate in diabetes and depletion of plasma bicarbonate with symptoms and physiological effects of alkali deficit becoming apparent.

In Tables 10 and 11 are shown the results of the analysis of bloods from fifteen different humans with diabetes mellitus. The blood sugar determinations were made in the Louisville City Hospital laboratory. Enough blood was taken from the patient to make possible a determination of organic acids and blood sugar at the same time. The diagnosis as indicated in the tables was taken from the patient's hospital chart. All but three of the patients had been receiving or were receiving insulin during the course of the experiment.

The data shows that there is no definite relation between high blood sugar and high total organic acids. To illustrate, case W. B. with an organic acid content of 44 milliequivalents and a blood sugar of 400 milligrams per 100 c.c. of blood, had a lower acid content than case G. H. whose blood sugar was approximately one half as much. Cases E. L. and M. T. had acid values which were normal or slightly above normal, but the blood sugars ranged from 280 to 353 which were

higher than in the case of E. P. where the blood sugar was less and the acid content greatly above normal. It appears from this data that the controlling factor is insulin.

It is known that in insulin therapy, the organic acids are removed from the blood by oxidation. This is very definitely demonstrated in the case B. B., G. C., M. C., and E. P. The first specimens of blood analyzed from these four cases gave the highest acid values, and, as subsequent analyses were made from week to week under insulin therapy, there was a very definite decrease in the organic acids. The blood sugar also decreased, but not in the same proportion as the organic acids.

The case of A. I. is interesting when compared with the four previously mentioned cases receiving insulin. This patient received no insulin during the five weeks course of the test. An inspection of the data shows that the organic acid and sugar content of the blood remained almost constant, even showing higher values for each at the end of the fifth week. Two other cases L. B. and E. S. received no insulin in treatment and, while their blood sugars were not over 162 and their cases were diagnosed as mild diabetes, yet the blood organic acids were 50 and 30 respectively. These acid values were fully as high or higher than similar values for cases diagnosed as moderately severe diabetes mellitus with acidosis, as in the cases of C. H. and A. H. It would seem that the effect of insulin therapy in removing organic acids from the blood was responsible for the lowered acid values even though the blood sugar values remained high.

SUMMARY

SUMMARY

1. The quinhydrone electrode method for the determination of organic acids in normal blood gave values comparable with those obtained by the slower and more difficult indirect method.

2. The concentration of organic acids in the blood of four pregnant women with clinical symptoms of eclampsia was no greater than in the blood of five normal, pregnant women.

3. The organic acid content of blood from nine individuals with pulmonary tuberculosis showed a marked increase. This increase was presumed to be due to the associated malnutrition.

4. Prolonged insulin therapy reduced the total organic acid content of blood in diabetes mellitus.

5. The total organic acid of diabetic blood was high in diabetes mellitus without insulin therapy, regardless of whether blood sugar values were high or low.

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